Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 1211–1221 **1211**
DOI: 10.1021/jm901545z

Transforming Rhinacanthin Analogues from Potent Anticancer Agents into Potent Antimalarial Agents

Ngampong Kongkathip,*[,†] Narathip Pradidphol,[†] Komkrit Hasitapan,[†] Ronald Grigg,[‡] Wei-Chun Kao,[§] Carola Hunte,[§] Nicholas Fisher,[∥] Ashley J. Warman,[∥] Giancarlo A. Biagini,[∥] Palangpon Kongsaeree,[⊥] Pitak Chuawong,[†] and Boonsong Kongkathip*[,†]

[†]*Natural Products and Organic Synthesis Research Unit (NPOS), Department of Chemistry and Center for Innovation in Chemistry, Faculty of Science, Kasetsart University, Jatujak, Bangkok 10900, Thailand,* [‡]*Molecular Innovation, Diversity and Automated Synthesis (MIDAS) Centre, School of Chemistry, University of Leeds, Leeds LS2 9JT, U.K.,* [§]*Institute of Membrane and Systems Biology, Astbury Center for Structural Molecular Biology, University of Leeds, Leeds LS2 9JT, U.K.,* [∥]*Liverpool School of Tropical Medicine, Liverpool L3 5QA, U.K., and* [⊥]*Department of Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Received October 19, 2009

Twenty-six novel naphthoquinone aliphatic esters were synthesized by esterification of 1,4-naphthoquinone alcohols with various aliphatic acids. The 1,4-naphthoquinone alcohols were prepared from 1-hydroxy-2-naphthoic acid in nine steps with excellent yields. Twenty-four of the novel synthetic naphthoquinone esters showed significant antimalarial activity with IC_{50} values in the range of 0.03−16.63 μM. The length of the aliphatic chain and the presence of C-2′ substituents on the propyl chain affected the activity. Interestingly, compounds **31** and **37** showed very good antimalarial activity and were not toxic to normal Vero cells, and the PTI values of **31** (> 1990.38) and **37** (1825.94) are excellent. Both **31** and **37** showed potent inhibition against *P. falciparum* 3D7 cyt bc_1 and no inhibition on rat cyt bc_1. They showed IC_{50} values in the nanomolar range, providing full inhibition of cyt bc_1 with one molecule inhibitor bound per cyt bc_1 monomer at the Q_o site.

Introduction

In our previous papers[1] we reported the syntheses of 1,2- and 1,4-naphthoquinones such as rhinacanthin-M (**1**), -N (**2a**), and -Q (**2b**) together with a substantial number of C-(3)-analogues thereof. These compounds are natural products isolated from the roots and leaves of *Rhincanthus nasutus* which are used in Thailand for the treatment of cancer.[2] Several of the novel analogues[1a] exhibited potent anticancer activity comparable to that of adriamycin (doxorubicin) (**3**) (Figure 1).

Quinones and, in particular, 1,4-naphthoquinones have long been known to display antimalarial activity[3] in addition to a wide variety of other bioactivities.[4] Pioneering work by Wendel[5] and by Fieser et al.[6] on hydroxynaphthoquinones formed the basis of the development of the antimalarial drug atovaquone (**4**, Figure 2, Figure 3),[7] while early clinical trials on **4** demonstrated good initial control of malaria, and currently **4** is used in combination with proguanil (**5**) under the trade name Malarone.[8] Atovaquone selectively inhibits the cytochrome bc_1 complex. The latter catalyzes electron transfer from ubiquinol to cytochrome *c* and concomitantly translocates protons across membranes.[9,10] A further consequence of inhibition of the cytochrome bc_1 complex is the indirect inhibition of pyrimidine biosynthesis.[11]

The structural similarities between rhinacanthin-C (**6**), -G (**7**), -H (**8**), and -K (**9**)[12] possessing C-(3)-aliphatic ester side chains and atovaquone suggested that rhinacanthin analogues might be a source of antimalarials. Our earlier work on anticancer agents focused on C-(3)-aromatic esters of rhinacanthins.[1a] We now report studies on C-(3)-aliphatic esters that have led to potent antimalarial compounds.

Chemistry

A total of 26 linear and branched aliphatic esters (Schemes 4 and 5 and Table 1) have been synthesized in our laboratory. All of these were prepared by coupling of the appropriate C-(3) aliphatic alcohol (**10a**−**c**) with linear and branched aliphatic carboxylic acids **11** to afford esters **12**[1] (Scheme 1). Naphthoquinone alcohol **10a** was synthesized starting from the commercially available 1-hydroxy-2-naphthoic acid in nine sequential steps[1a] with excellent yields (Scheme 2). Naphthoquinone aliphatic esters (**18**−**39**) were synthesized from naphthoquinone alcohol **10a** and various aliphatic acids by DCC/DMAP[a] coupling in moderate to good yields (Table 1). Naphthoquinone alcohol **10b** could be obtained in good yields via six steps[1b] by using 1-naphthol as starting material (Scheme 3). Esterification of **10b** with acetic acid, butyric acid, and octanoic acid using CDI as a condensing agent afforded naphthoquinone esters **42**−**44** in moderate yields (Scheme 4). The cyclohexyl alcohol **10c** was prepared from **22** by ring-opening using 1% aqueous sodium hydroxide, and then naphthoquinone ester **48** was subsequently obtained by coupling **10c** with octanoic acid using DCC in the presence of a catalytic amount of DMAP (Scheme 5).

*To whom correspondence should be address. For N.K.: phone, +6625625555, extension 2139; fax, +6625793955; e-mail, fscinpk@ku.ac.th. For B.K.: phone, +6625625555, extension 2235; fax, +6625793955; e-mail, fscibsk@ku.ac.th.

[a]Abbreviations: DCC, *N*,*N*′-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; CDI, 1,1′-carbonyldiimidazole; Q_o site, quinol oxidizing site of bc_1 complex; Q_i site, quinone reducing site of bc_1 complex; DQH, decylubiquinol; YPD, yeast peptone dextrose; EDTA, ethylenediaminetetraacetic acid.

Published on Web 01/12/2010

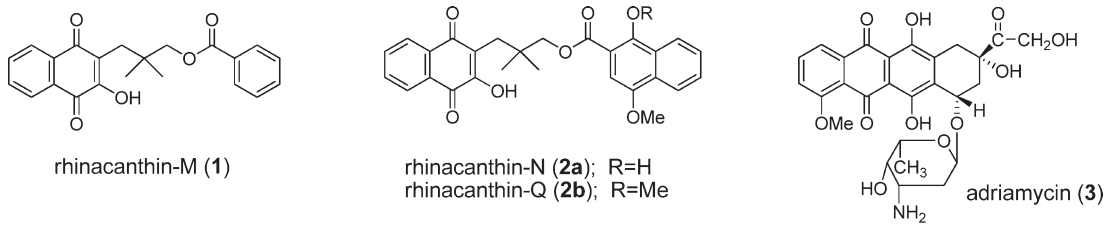


Figure 1. Structures of rhinacanthin-M (**1**), -N (**2a**), -Q (**2b**), and adriamycin (**3**).

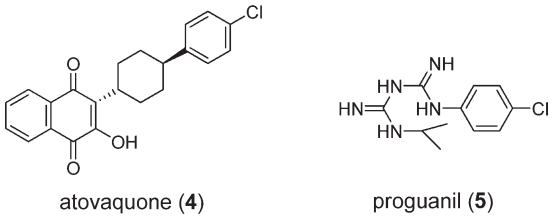


Figure 2. Structures of atovaquone (**4**) and proguanil (**5**).

Antimalarial Activity

The synthetic 1,4-naphthoquinone aliphatic esters (**18**−**39**, **42**−**44**, and **46**) were evaluated for antimalarial activity using dihydroartemisinin as reference (Tables 2 and 3). *Plasmodium falciparum* (K1, multidrug resistant strain) was cultivated in vitro according to Trager and Jensen.[13a] Quantitative assessment of antimalarial activity in vitro was determined by microculture radioisotope techniques based upon the methods described by Desjardins.[13b]

Cytotoxicity

Compounds **18**−**39** were subjected to cytotoxic evaluation against the normal Vero cell lines employing the MTT colorimetric method.[14] The results are summarized in Table 2.

Activity of cyt bc_1

With highly potent antimalarial activity and no cytotoxicity against Vero cell lines, naphthoquinone esters **31** and **37** were further tested for their inhibition of cyt bc_1 of *P. falciparum* 3D7, yeast, rat, and purified yeast cyt bc_1 by using decylubiquinol-linked cytochrome *c* reductase assay. The results are shown in Tables 4 and 5.

Decylubiquinol/Cytochrome *c* Reductase Assay

Cytochrome bc_1 activity[15] was monitored spectrophotometrically in *P. falciparum* cell-free extracts and yeast mitochondrial membrane preparations by the steady-state decylubiquinol/cytochrome *c* reductase.

Results and Discussion

The prepared 1,4-naphthoquinone aliphatic esters exhibited significant antimalarial activity (Tables 2 and 3). Esters with short chains (n = 1, 2) showed less activity than those with longer straight chains. Compounds **26** (n = 4), **29** (n = 5), **30** (n = 6), **31** (n = 7), **33** (n = 8), **34** (n = 9), **35** (n = 10), and **36** (n = 11) showed quite strong antimalarial activity. Among the compounds with linear aliphatic chain (n = 1−11), compound **31** (n = 7) showed the best activity and had no toxicity against normal Vero cells corresponding to a favorably very high potential therapeutic index (PTI > 1990). The length of the aliphatic chain of this compound is the same as that of natural rhinacanthin-C (**6**), -G (**7**), -H (**8**), and -K (**9**). The naphthoquinone aliphatic ester **39** (n = 17) (IC_{50} = 0.133 μM) with quite longer chain than n = 11 showed similar activity as compound **31** (n = 7) (IC_{50} = 0.13 μM), whereas compounds **37** (n = 13) and **38** (n = 15) showed the best activity with favorable IC_{50} values of 0.032 and 0.030 μM, respectively. In particular, compound **37** showed very high PTI (1826). It seems that the optimum length of the aliphatic linear chain is n = 13 and 15.

Because most of the natural naphthoquinones contain an α-methyl substituent on the aliphatic ester side chain, compounds **20**, **22**, **27**, and **32** with α-methyl substituents were synthesized (as racemates) by the same method as mentioned above. The test results showed that these naphthoquinone esters showed stronger activity than the compounds containing linear chains of the same number of carbons (IC_{50} = 0.11−0.91 μM). Moreover, activity was enhanced 2-fold in compound **25** with the *S*-configuration of the methyl substituent (IC_{50} = 0.11 μM) compared with that of the racemic mixture **22** (IC_{50} = 0.22 μM).

While an α-methyl substituent enhanced the activity, compounds **23** and **28** with β-methyl substituents showed reduced activity. Compound **23** showed about 2 times less activity than **22**. An α-ethyl substituent, as in compound **24**, also caused reduced activity, compared with compound **22** (α-methyl substituent). Compounds lacking gem-dimethyl substitution in the propyl chain attached to the 1,4-naphthoquinone

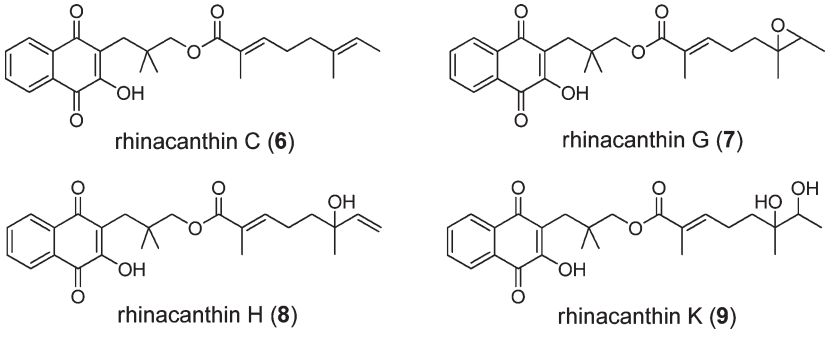
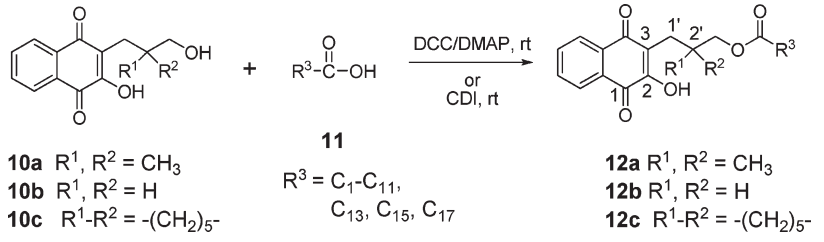
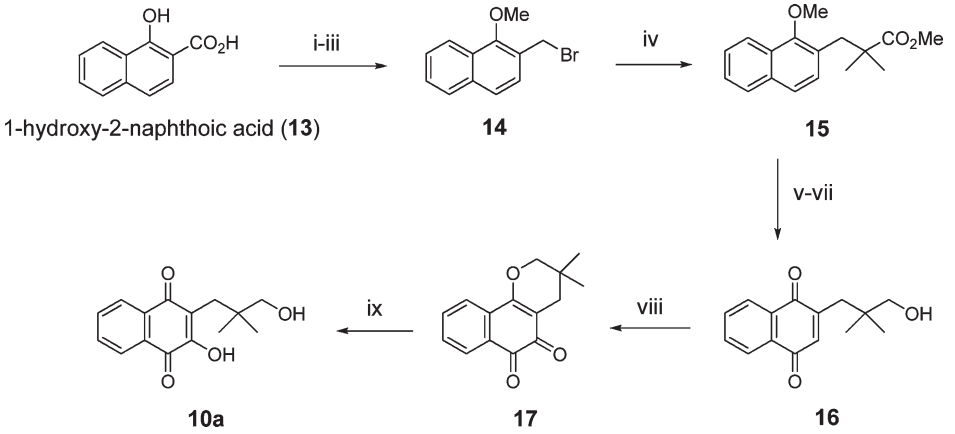


Figure 3. Structures of rhinacanthin-C (**6**), -G (**7**), -H (**8**), and -K (**9**).

Scheme 1. Esterification of Naphthoquinone Alcohols **10a**−**c** with Various Aliphatic Acids

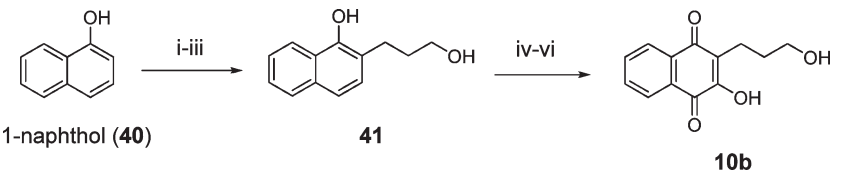


Scheme 2. Synthesis of Naphthoquinone Alcohol **10a**[a]



[a] Reagents and conditions: (i) MeI, K_2CO_3, acetone, reflux, 96%; (ii) $LiAlH_4$, THF, 0 °C to room temp, 94%; (iii) 1 M PBr_3 in CH_2Cl_2, CH_2Cl_2, room temp, 100%; (iv) methyl isobutyrate, LDA, THF, −78 °C, 89%; (v) $AlCl_3$, PhCl, reflux, 92%; (vi) $LiAlH_4$, THF, 0 °C to room temp, 88%; (vii) Fremy's salt, MeOH/DMF (3:1), 1 M NaOAc, room temp, 83%; (viii) DDQ, *p*-TsOH, PhH, reflux, 90%; (ix) 1% aq NaOH, reflux, 84%.

Scheme 3. Synthesis of Naphthoquinone Alcohol **10b**[a]



[a] Reagents and conditions: (i) allyl bromide, K_2CO_3, reflux, 86%; (ii) 180 °C, DMF, 87%; (iii) $BH_3 \cdot THF$, then H_2O_2/NaOH, 79%; (iv) Fremy's salt, MeOH/DMF (3:1), 1 M NaOAc, room temp, 83%; (v) DDQ, *p*-TsOH, PhH, reflux, 93%; (vi) 1% aq NaOH, reflux, 89%.

nucleus, as in **42** and **43**, were inactive, whereas **44** (IC_{50} = 5.15 μM) and compound **46**, having a spiro C-2′ cyclohexyl group (IC_{50} = 2.63 μM), showed only reduced activity. Thus C-2′ gem-dimethyl substitution of the propyl chain is favorable for good activity in this series of esters.

We have found that some of our compounds such as **31** and **37** showed very potent antimalarial activity but were not toxic to Vero cells, so **31** and **37** were further tested in vivo as well as the mechanism of their antimalarial activity. As a known atovaquone, one of 1,4-naphthoquinone, has been reported to inhibit the cyt bc_1 of *P. falciparum*, this inhibition may be a mechanism of our synthesized antimalarial naphthoquinones. So we investigated the inhibition mechanism of compounds **31** and **37** by decylubiquinol/cytochrome *c* reductase assay. Compounds **31** and **37** were determined for their cyt bc_1 activity using stigmatellin as reference. Compound **31** inhibited *P. falciparum* 3D7, yeast, and rat with IC_{50} values of 5.4 ± 0.53, 16 ± 1.30, and 3,000 ± 156 nM, respectively. Compound **37** also showed inhibition with IC_{50} values of 8.0 ± 0.60, 79.6 ± 3.41, and 2,495 ± 820 nM, respectively (Table 4). Our test showed IC_{50} of stigmatellin as 9.1 nM (Table 5), in agreement with previous data.[16] IC_{50} values of **31** and **37** were determined as 840 and 770 nM, respectively, in detergent environment. These inhibitors **31** and **37** apparently have lower affinities than stigmatellin, explaining the 50 times higher IC_{50} values in detergent environment compared to those in the membrane because binding of the hydrophobic compounds competes with binding to detergent micelles.

Inhibitor binding to Q_o vs Q_i site of cyt bc_1 was distinguished by DQH induced redox difference spectra in the presence of specific inhibitors. Stigmatellin binds specifically to the Q_o site while antimycin A inhibits cyt bc_1 by occluding the electron transfer from heme b_H to the quinone substrate in the Q_i site.[17] Both inhibitors (**31** and **37**) have IC_{50} values in the nanomolar range, providing full inhibition of cyt bc_1 with one molecule inhibitor bound per cyt bc_1 monomer. Spectra of DQH reduced inhibitor−cyt bc_1 complex differ for Q_o and Q_i site inhibitors at the absorbance for heme c_1 at 553.6 nm (Figure 4). Stigmatellin blocks electron transfer from the Q_o site to the iron sulfur cluster; thus, very limited heme c_1 was reduced (Figure 4, upper panel) by 45 μM DQH. However, heme c_1 was fully reduced in the presence of antimycin A

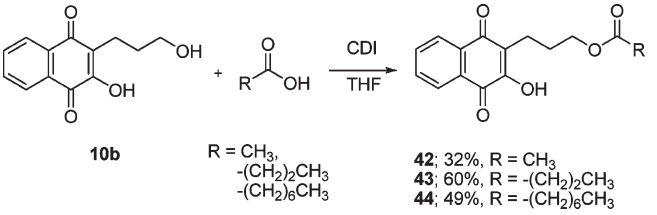
(Figure 4, middle panel). Redox difference spectra quantification ($\varepsilon_{562-575nm}$) showed that one (0.9) of two *b* hemes were reduced by DQH, indicating that one DQH was oxidized per one cyt bc_1 monomer as expected. The interaction site of compounds **31** and **37** was determined by comparison with these standards. Very limited heme c_1 was reduced by 45 μM DQH in both **31** and **37** cyt bc_1 complexes with AU $\leq$ 0.02 indicative for blocked electron transfer to the high potential chain as observed for the stigmatellin-inhibited complex, thus clearly revealing both **31** and **37** binding to the Q_o site (Figure 4, lower panel, solid and dotted lines). Interestingly, less cytochrome *b* is reduced for the **31** and **37** probes (0.02 AU) compared to stigmatellin (0.05 AU), indicating that these inhibitors may also bind to a certain extent to the Q_i site.

The Q_o site has been the target of both antimalarial drug development and of agrochemical fungicides. Both applications have suffered from the development of resistance arising from site mutations of the bc_1 complex.[18,19] Considerable detailed evidence has accrued about the nature of these mutations which will assist further drug development programs. Very recently the Q_o has also been proposed as the site of action of the anticancer macrolides leucascandrolide A and neopentolide[20] and for the fungicidal crocacins A and D.[21] Currently there is no single marketed drug that is effective for the treatment of multidrug resistant malarial parasites. However, recent disclosures of the metabolism and pharmacokinetics of GW844520 and other related 4-pyridone antimalarials indicate that this class of compounds acts by selective inhibition of mitochondrial electron transport at the cytochrome bc_1 complex involving the Q_o site.[22,23] In this case no significant cross-resistance with atovaquone resistant strains of *P. falciparum* is observed and the frequency at which resistant strains arise in vitro is significantly lower than that displayed by atovaquone. These observations indicate there are significant opportunities for further exploration of the Q_o site. Thus, it is of interest to see whether the naphthoquinone ester system can be further developed to produce a new antimalarial drug.

However, since our naphthoquinone esters affect cyt bc_1 similar to atovaquone, it is our concern that the activity of these newly synthesized naphthoquinone esters toward atovaquone resistance *P. falciparum* might be different. And also the structural differences between our naphthoquinone esters and atovaquone, i.e., aliphatic ester moiety of our naphthoquinone versus a cyclohexyl unit of atovaquone, should render different binding affinity toward cytochrome bc_1 protein in *P. falciparum*. It is conceivable to anticipate that our naphthoquinone esters would impart resistant in *P. falciparum*. It is worth mentioning here that several antimalarial drug resistance cases caused by mutations at other drug targets have also been reported.[24,25] To circumvent this problem, a drug combination that acts on the principle of synergy might avoid this problem as advocated by White.[26] In the case of our compounds we believe our ester **37** given in combination with our reference drug dihydroartimisinin will be effective. Dihydroartemisinin is the common metabolite of artemisinin and its derivatives, artemether and artesunate. These are the most active of all antimalarial drugs.[26]

From our naphthoquinone basic structure, it would be said that four functional parts showed important roles, which are (i) 2′-substituents of propyl chain, (ii) the chain length of aliphatic chain, (iii) α-methyl substituent of aliphatic chain, (iv) ester moiety. Synthesis of the modified antimalarial naphthoquinones is being investigated by our group which could be helpful in designing future antimalarial drugs caused by mitochondria-containing parasite.

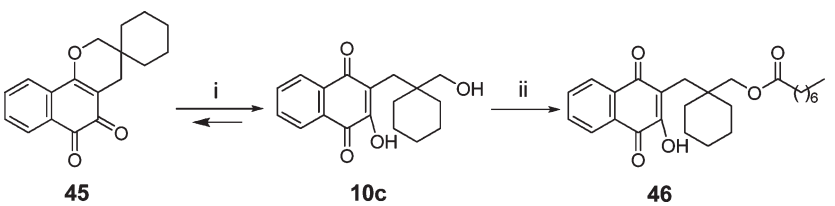
Scheme 4. Esterification of **10b** with Aliphatic Carboxylic Acids Using CDI as a Condensing Agent



Conclusion

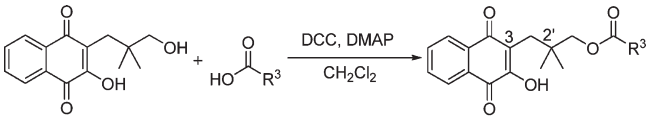
Twenty-six novel 1,4-naphthoquinone aliphatic esters were synthesized, and twenty-four of them exhibited potent antimalarial activity,[27] especially compounds **22**, **25**, **27**, **31**, **32**, **37**, **38**, and **39** (IC_{50} = 0.03−0.22 μM). The α-methyl substituent of the acid side chain affected the antimalarial activity. Naphthoquinone aliphatic esters with α-methyl substituent of the side chain were more active than those with β-methyl substituent and the linear side chain with the same number of carbon atoms. In addition, α-ethyl substituent showed less activity than α-methyl substituent. C-2′ Substituents of the propyl side chain at the 3-position of the naphthoquinone nucleus showed some effect on the activity. The C-2′ dimethyl group showed more potent activity than that with no substituent or with C-2′ cyclohexyl group. Most of our naphthoquinones showed no toxicity on normal Vero cell lines as well as very good antimalarial activity. Compounds **31** and **37** showed antimalarial activity with IC_{50} values of 0.13 and 0.032 μM, respectively, whereas they showed no toxicity to Vero cell lines (IC_{50} values of >258.75 and 58.43 μM, respectively), corresponding to excellent PTI values of >1990 and 1826, respectively. Compounds **31** and **37** showed potent inhibition against *P. falciparum* 3D7 cyt bc_1 with IC_{50} values of 5.4 ± 0.53 and 8.0 ± 0.60 nM, respectively, while they showed less inhibition on yeast cyt bc_1 (IC_{50} = 16 ± 1.30 and 79.6 ± 3.41 nM, respectively) and no inhibition on rat cyt bc_1 (IC_{50} = 3,000 ± 156 and 2,495 ± 820 nM, respectively). Both **31** and **37** showed IC_{50} values in the nanomolar range, providing full inhibition of

Scheme 5. Synthesis of Cyclohexyl Alcohol **10c** and Naphthoquinone Ester **46**[a]



[a] Reagents and conditions: (i) 1% aq NaOH, reflux; (ii) octanoic acid, DCC, DMAP, CH_2Cl_2, room temp, 35% over two steps.

Table 1. Esterification of **10a** with Aliphatic Carboxylic Acids Using DCC/DMAP as a Condensing Agent



10a **18-39**

Entry	R^3	Naphthoquinone ester	Yield (%)
1	$-CH_3$	**18**	89
2	$-CH_2-CH_3$	**19**	90
3	$-CH(CH_3)-CH_3$	**20**	86
4	$-(CH_2)_2-CH_3$	**21**	87
5	$-CH(CH_3)-CH_2-CH_3$	**22**	81
6	$-CH_2-CH(CH_3)-CH_3$	**23**	82
7	$-CH(CH_2-CH_3)-CH_2-CH_3$	**24**	73
8	$-CH(CH_3)-CH_2-CH_3$	**25**	76
9	$-(CH_2)_3-CH_3$	**26**	45
10	$-CH(CH_3)-(CH_2)_2-CH_3$	**27**	77
11	$-CH_2-CH(CH_3)-CH_2-CH_3$	**28**	76
12	$-(CH_2)_4-CH_3$	**29**	74
13	$-(CH_2)_5-CH_3$	**30**	83
14	$-(CH_2)_6-CH_3$	**31**	96
15	$-CH(CH_3)-(CH_2)_5-CH_3$	**32**	84
16	$-(CH_2)_7-CH_3$	**33**	73
17	$-(CH_2)_8-CH_3$	**34**	72
18	$-(CH_2)_9-CH_3$	**35**	83
19	$-(CH_2)_{10}-CH_3$	**36**	79
20	$-(CH_2)_{12}-CH_3$	**37**	80
21	$-(CH_2)_{14}-CH_3$	**38**	77
22	$-(CH_2)_{16}-CH_3$	**39**	74

cyt bc_1 with one molecule inhibitor bound per cyt bc_1 monomer. Therefore, it is clear that our naphthoquinone esters **31** and **37** revealed their binding to the Q_o site. It is not far-fetched to think that inhibition of *P. faciparum* is an important attribute of antimalarial activity; thus, it is of interest to see whether the naphthoquinone ester system can be developed sufficiently to produce a new antimalarial drug.

Experimental Section

General Remarks. Melting points were determined on a Fisher John apparatus and are uncorrected. The IR spectra were recorded on an FTIR Perkin-Elmer system 2000. Mass spectral data were obtained on the GCMS-QP-5050A. Nuclear magnetic resonance spectra were recorded at 400 MHz on a Bruker Advance DPX-400. Chemical shifts (δ) are given in parts per million downfield from tetramethylsilane (TMS) as internal standard. Coupling constants are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, m = multiplet, dd = doublet of doublet. Elemental analyses were performed for compounds **18**−**39**, **42**−**44**, and **46** on an elemental analyzer (EA), Perkin-Elmer PE2400 series II (Scientific and Technological Research Equipment Centre, Chulalongkorn University, Bangkok, Thailand), and the results are within ±0.4% of the theoretical values and indicate >95% purity; values are presented in the Supporting Information. Column chromatography was performed with flash silica gel (Merck 9385).

General Procedure for Synthesis of Naphthoquinone Aliphatic Esters 18−39. A solution of naphthoquinone alcohol (0.2 mmol) in dry dichloromethane (2 mL) was added to a stirred solution of carboxylic acid (0.26 mmol) and 4-dimethylaminopyridine (DMAP) (0.06 mmol) in dry dichloromethane (2 mL). The reaction mixture was stirred at room temperature for 5 min when a solution of 1,3-dicyclohexylcarbodiimide (DCC) (0.26 mmol) in dry dichloromethane (3 mL) was added. Stirring was continued overnight at room temperature. The precipitate of dicyclohexylurea was filtered off and the filtrate washed with saturated ammonium chloride solution, water, dried (anhydrous Na_2SO_4), and filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography.

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Acetate (18). Flash column chromatography was used, eluting with 5% ethyl acetate−hexane. The yellow oil was obtained in 89% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 113.5−114.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.92 (s, 6H, 2 × CH_3), 1.95 (s, 3H, $COCH_3$), 2.6 1 (s, 2H, CH_2Ar), 3.77 (s, 2H, OCH_2), 7.40 (s, 1H, OH), 7.62 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.02 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.05 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Propionate (19). Flash column chromatography was used, eluting with 2% ethyl acetate−hexane. The yellow oil was obtained in 90%, and then it was crystallized with hexane−dichloromethane to yield yellow needles, mp 89.0−90.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (s, 6H, 2 × CH_3), 1.13 (t, J = 7.6 Hz, 3H, CH_2-$\underline{CH_3}$), 2.31 (q, J = 7.6 Hz, 2H, $\underline{CH_2}$-CH_3), 2.69 (s, 2H, CH_2Ar), 3.86 (s, 2H, OCH_2), 7.43 (s, 1H, OH), 7.70 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 8.13 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Isobutyrate (20). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 86%, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 70.5−71.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.99 (s, 6H, 2 × CH_3), 1.17 (d, J = 7.0 Hz, 6H, ($\underline{CH_3}$)$_2$-CH), 2.54 (sept, 1H, (CH_3)$_2$-$\underline{CH}$), 2.69 (s, 2H, CH_2Ar), 3.85 (s, 2H, OCH_2), 7.44 (s, 1H, OH), 7.70 (td, J = 7.5 Hz, J = 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.5 Hz, J = 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.5 Hz, J = 1.2 Hz, 1H, ArH), 8.13 (dd, J = 7.5 Hz, J = 1.2 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Butyrate (21). Flash column chromatography was used, eluting with 7% ethyl acetate−hexane. The yellow oil was obtained in 87% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 66.0−67.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85 (t, J = 7.4 Hz, 3H, $\underline{CH_3}$-CH_2), 0.92 (s, 6H, 2 × CH_3), 1.56 (m, 2H, CH_3-$\underline{CH_2}$-CH_2), 2.18 (t, J = 7.4 Hz, 2H, CH_2-$\underline{CH_2}$-COO), 2.61 (s, 2H, CH_2Ar), 3.79 (s, 2H, OCH_2), 7.37 (s, 1H, OH), 7.62 (td, J = 7.6 Hz, J = 1.4 Hz, 1H,

Table 2. Antimalarial Activity and Cytotoxicity against Vero Cell Lines of Naphthoquinone Esters (**18**−**39**)

Compound	Acid (R^3-)	Number of carbon atoms of straight chain (n)	Antimalarial activity, IC_{50}[a] (μM)	Vero cell,[b] IC_{50} (μM)[c]
18	—CH_3	1	10.92	138.92±22.13
19	—CH_2-CH_3	2	4.1	60.06±7.74
20	—CH(-CH_3)-CH_3	2	0.91	123.10±50.52
21	—$(CH_2)_2$-CH_3	3	0.91	73.16±14.89
22	—CH(-CH_2-CH_3)-CH_3	3	0.22	0.87±0.02
23	—CH_2-CH(-CH_3)-CH_3	3	0.41	0.55
24	—CH(-CH_2-CH_3)-CH_2-CH_3	3	16.63	104.43
25	—CH(-CH_2-CH_3)-CH_3	3	0.11	0.57±0.015
26	—$(CH_2)_3$-CH_3	4	0.58	7.84±0.67
27	—CH(-$(CH_2)_2$-CH_3)-CH_3	4	0.12	0.12±0.03
28	—CH_2-CH(-CH_2-CH_3)-CH_3	4	0.19	2.30
29	—$(CH_2)_4$-CH_3	5	1.12	169.07±15.60
30	—$(CH_2)_5$-CH_3	6	0.81	99.34±7.36
31	—$(CH_2)_6$-CH_3	7	0.13	>258.75
32	—CH(-$(CH_2)_5$-CH_3)-CH_3	7	0.11	0.45±0.02
33	—$(CH_2)_7$-CH_3	8	0.50	149.81±13.73
34	—$(CH_2)_8$-CH_3	9	0.72	202.64±36.16
35	—$(CH_2)_9$-CH_3	10	0.70	>233.34
36	—$(CH_2)_{10}$-CH_3	11	0.90	248.54±101.67
37	—$(CH_2)_{12}$-CH_3	13	0.032	58.43±5.82
38	—$(CH_2)_{14}$-CH_3	15	0.030	4.41±2.77
39	—$(CH_2)_{16}$-CH_3	17	0.133	15.51±1.56
Dihydroartemisinin[d]	-		0.0035	-

[a] IC_{50} = half-maximal inhibitory concentration. [b] Vero cell lines = African green monkey kidney cell. [c] The results are the mean of six replicate determinations ± SD. [d] Used as antimalarial reference.

ArH), 7.69 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.02 (m, 1H, ArH), 8.05 (m, 1H, ArH).

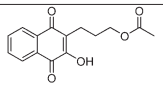
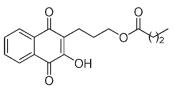
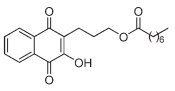
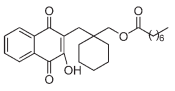
3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl 2-Methylbutanoate (22). Flash column chromatography was used, eluting with 4% ethyl acetate−hexane. The yellow gum was obtained in 81% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (t, J=7.2 Hz, 3H, $\underline{CH_3}CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.14 (d, J=7.2 Hz, 3H, $\underline{CH_3}CH(CO)$), 1.46 (m, 1H, $\underline{CH_2}CH_3$), 1.70 (m, 1H, $\underline{CH_2}CH_3$), 2.35 (hextet, J = 6.8 Hz, 1H, $\underline{CH_3}\underline{CH}$-(CO)), 2.69 (s, 2H, CH_2Ar), 3.87 (s, 2H, OCH_2), 7.45 (s, 1H, OH), 7.70 (td, J=7.5 Hz, J=1.2 Hz, 1H, ArH), 7.77 (td, J=7.5 Hz, J = 1.2 Hz, 1H, ArH), 8.10 (ddd, J = 7.5 Hz, J = 1.2 Hz, J = 0.6 Hz, 1H, ArH), 8.13 (ddd, J = 7.5 Hz, J = 1.2 Hz, J = 0.6 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Isovalerate (23). Flash column chromatography, eluting with 5% ethyl acetate−hexane, gave a yellow oil (82%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85 (d, J = 6.6 Hz, 6H, 2 × CH_3), 0.92 (s, 6H, 2 × CH_3), 2.00 (m, 1H, $CH(CH_3)_2$), 2.08 (m, 2H, $CH_2C(O)OR$), 2.61 (s, 2H, CH_2Ar), 3.79 (s, 2H, $CH_2OC(O)R$), 7.37 (s, 1H, OH), 7.62 (td, J = 7.5 Hz, J = 1.4 Hz, 1H, ArH), 7.70 (td, J = 7.5 Hz, J = 1.4 Hz, 1H, ArH), 8.02 (m, 1H, ArH), 8.06 (m, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl 2-Ethylbutanoate (24). Flash column chromatography, eluting with 2% ethyl acetate−hexane, gave a yellow oil (73%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80 (t, J = 7.4 Hz, 6H, 2 × CH_3), 0.92 (s, 6H, 2 × CH_3), 1.43 (m, 2H, CH_2CH_3), 1.53 (m, 2H, CH_2CH_3), 2.11 (m, 1H, $CH_2C(O)OR$), 2.62 (s, 2H, CH_2Ar), 3.81 (s, 2H, $CH_2OC(O)R$), 7.38 (s, 1H, OH), 7.62 (td, J = 7.5 Hz, J=1.4 Hz, 1H, ArH), 7.69 (td, J=7.5 Hz, J=1.4 Hz, 1H, ArH), 8.02 (m, 1H, ArH), 8.06 (m, 1H, ArH).

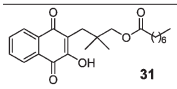
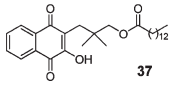
(*S*)-3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl 2-Methylbutanoate (25). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow gum was obtained in 76% yield. ^{1}H NMR ($CDCl_3$,

Table 3. Antimalarial Activity of Naphthoquinone Esters (**42**−**44** and **46**)

Compound	Structure	Antimalarial activity, IC_{50}[a] (μM)[b]
42		inactive
43		inactive
44		5.15
46		2.63
Dihydroartemisinin[c]		0.0035

[a] IC_{50} = half-maximal inhibitory concentration. [b] The results are the mean of six replicate determinations. [c] Used as antimalarial reference.

Table 4. Inhibition of *P. falciparum* 3D7, Yeast, and Rat cyt bc_1 by **31** and **37**

Compound	IC_{50}[a] bc_1 inhibition[b] (nM)		
	P. falciparum 3D7	Yeast	Rat
 **31**	5.4 ± 0.53	16 ± 1.30	3,000 ±156
 **37**	8.0 ± 0.60	79.6 ± 3.41	2,495 ± 820

[a] IC_{50} = half-maximal inhibitory concentration. IC_{50} values (presented as ±SEM) were obtained from three separate assays and calculated from four-parameter sigmoidal fits. [b] The cyt bc_1 activity was monitored spectrophotometrically as decylubiquinol-linked cytochrome *c* reduction assay.

Table 5. Inhibition of Activity of Purified Yeast cyt bc_1 in Detergent by Stigmatellin, **31**, and **37**

	stigmatellin	**31**	**37**
IC_{50}[a]	9.1 nM	0.84 μM	0.77 μM

[a] IC_{50} = half-maximal inhibitory concentration. IC_{50} values were calculated from logarithmic fits, and the mean value of two separate assays is given.

400 MHz) δ: 0.89 (t, J = 7.2 Hz, 3H, $\underline{CH_3}CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.14 (d, J = 7.2 Hz, 3H, $\underline{CH_3}CH(CO)$), 1.46 (m, 1H, $\underline{CH_2}CH_3$), 1.67 (m, 1H, $\underline{CH_2}CH_3$), 2.35 (hextet, J = 7.6 Hz, 1H, $CH_3\underline{CH}(CO)$), 2.69 (s, 2H, $\underline{CH_2}Ar$), 3.87 (s, 2H, OCH_2), 7.44 (s, 1H, $\underline{OH}$), 7.70 (td, J = 7.6 Hz, J = 1.6 Hz, 1H, ArH), 7.78 (td, J = 7.6 Hz, J = 1.6 Hz, 1H, ArH), 8.10 (m, 1H, ArH), 8.13 (m, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Pentanoate (26). Flash column chromatography was used, eluting with % ethyl acetate−hexane. The yellow oil was obtained in 45% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 51.0−52.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, J = 7.3 Hz, 3H, $\underline{CH_3}CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.32 (m, 2H, $CH_3\underline{CH_2}CH_2$), 1.58 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.27 (t, J = 7.6 Hz, 2H, $COCH_2\underline{CH_2}$), 2.68 (s, 2H, $\underline{CH_2}Ar$), 3.85 (s, 2H, OCH_2), 7.44 (s, 1H, $\underline{OH}$), 7.69 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 7.67 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.09 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.13 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl-2-methylpentanoate (27). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 77% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 57.0−58.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, J = 7.2 Hz, 3H, $\underline{CH_3}CH_2CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.14 (d, J = 7.2 Hz, 3H, $\underline{CH_3}CH(CO)$), 1.24−1.42 (m, 3H, $\underline{CH_2}CH_2CH_3$), 1.65 (m, 1H, $\underline{CH_2}CH_2CH_3$), 2.43 (hextet, J = 7.2 Hz, 1H, $CH_3\underline{CH}(CO)$), 2.69 (s, 2H, $\underline{CH_2}Ar$), 3.86 (s, 2H, OCH_2), 7.45 (br s, 1H, OH), 7.70 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 7.77 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 8.10 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 8.14 (dd, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl 3-Methylpentanoate (28). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow gum was obtained in 76% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.79 (t, J = 7.4 Hz, 3H, CH_3-CH_2), 0.83 (d, J = 6.9 Hz, 3H, CH_3-CH), 0.92 (s, 6H, 2 × CH_3), 1.77 (m, 1H, CH), 2.00 (dd, J = 14.7 Hz, J = 7.1 Hz, 1H, $C(O)CH_2CH$), 2.19 (dd, J = 14.7 Hz, J = 7.1 Hz, 1H, $C(O)CH_2CH$), 2.61 (s, 2H, CH_2Ar), 3.79 (s, 2H, $CH_2OC(O)R$), 7.37 (s, 1H, OH), 7.62 (td, J = 7.5 Hz, J = 1.4 Hz, 1H, ArH), 7.69 (td, J = 7.5 Hz, J = 1.4 Hz, 1H, ArH), 8.02 (ddd, J = 7.5 Hz, J = 1.4 Hz, J = 0.4 Hz, 1H, ArH), 8.06 (ddd, J = 7.5 Hz, J = 1.4 Hz, J = 0.4 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Hexanoate (29). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 74% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 54.5−55.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.89 (t, J = 6.7 Hz, 3H, $\underline{CH_3}CH_2$), 1.00 (s, 6H, 2 × CH_3), 1.28 (m, 4H, $(\underline{CH_2})_2CH_3$), 1.51 (quin, J = 7.5, 2H, $COCH_2\underline{CH_2}CH_2$), 2.27 (t, 2H, J = 7.5 Hz, $CO\underline{CH_2}CH_2$), 2.69 (s, 2H, $\underline{CH_2}Ar$), 3.87 (s, 2H, OCH_2), 7.62 (br s, 1H, OH), 7.69 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 7.77 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.08 (dd, 1H, J = 7.6 Hz, J = 1.4 Hz, ArH), 8.12 (dd, 1H, J = 7.6 Hz, J = 1.4 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Heptanoate (30). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 83% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 57.5−58.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, J = 6.9 Hz, 3H, $\underline{CH_3}CH_2$), 1.00 (s, 6H, 2 × CH_3), 1.28 (m, 6H, $(\underline{CH_2})_3CH_2$), 1.60 (quin, J = 7.4 Hz, 2H, $COCH_2\underline{CH_2}CH_2$), 2.28 (t, 2H, J = 7.4 Hz, $CO\underline{CH_2}CH_2$), 2.69 (s, 2H, $\underline{CH_2}Ar$), 3.87 (s, 2H, OCH_2), 7.58 (br s, 1H, OH), 7.70 (td, J = 7.6 Hz, J = 1.1 Hz, 1H, ArH), 7.78 (td, J = 7.6 Hz, J = 1.1 Hz, 1H, ArH), 8.10 (dd, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH), 8.13 (dd, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Octanoate (31). Flash column chromatography was used, eluting with 5% ethyl acetate−hexane. The yellow oil was obtained in 96% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 56.0−57.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.87 (t, J = 6.9 Hz, 3H, $\underline{CH_3}CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.27 (br s, 8H, CH_3-$(\underline{CH_2})_4CH_2$), 1.59 (m, 2H, $COCH_2\underline{CH_2}CH_2$), 2.26 (t, J = 7.6 Hz, 2H, $CO\underline{CH_2}CH_2$), 2.68 (s, 2H, $\underline{CH_2}Ar$), 3.85 (s, 2H, OCH_2), 7.44 (s, 1H, $\underline{OH}$), 7.69 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 7.76 (td, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.09 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH), 8.12 (dd, J = 7.6 Hz, J = 1.4 Hz, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl-2-methyl Octanoate (32). Flash column chromatography was used, eluting with 4% ethyl acetate−hexane. The yellow gum was obtained in 84% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.86 (t, J = 6.8 Hz, 3H, $\underline{CH_3}CH_2CH_2$), 0.99 (s, 6H, 2 × CH_3), 1.13 (d, J = 7.0 Hz, 3H, $\underline{CH_3}CH(CO)$), 1.25 (m, 6H, CH_2-$\underline{CH_2}CH_2CH_2CH_3$), 1.37 (m, 2H, $CH_3CH(CO)CH_2\underline{CH_2}CH_2$), 1.64 (m, 2H, $CH_3CH(CO)\underline{CH_2}CH_2CH_2$), 2.40 (hextet, J = 7.0 Hz, 1H, $CH_3\underline{CH}(CO)CH_2$), 2.68 (s, 2H, CH_2Ar), 3.85 (s, 2H,

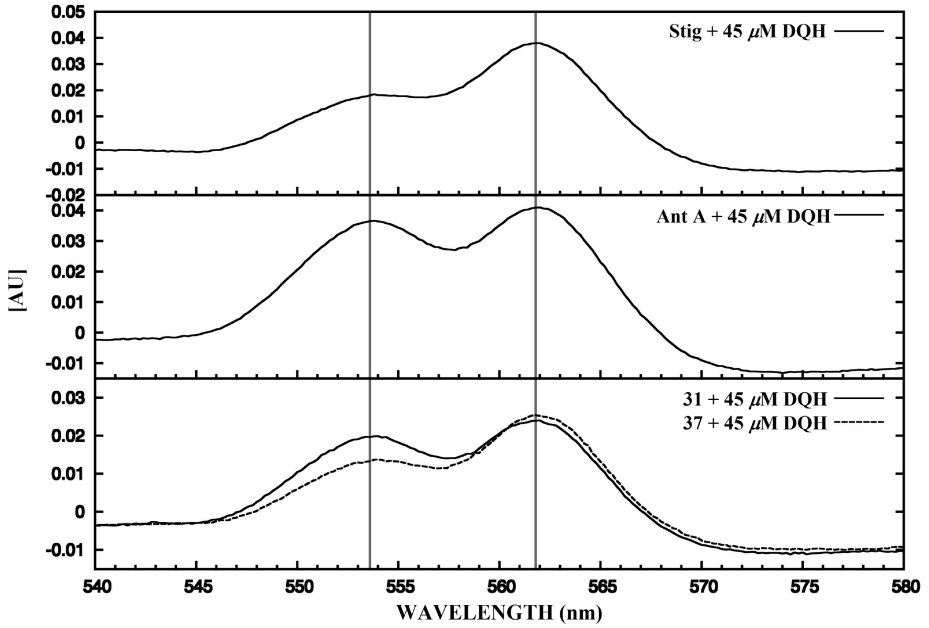


Figure 4. Redox difference spectra of purified yeast cyt bc_1 in the presence of inhibitors (stigmatellin (Stig), antimycin A (Ant A), **31**, and **37**). Spectra were calculated as DQH-reduced cyt bc_1 complex minus inhibitor−cyt bc_1 complex. The cyt bc_1 at 2 μM was used for each assay. Vertical lines indicate the relevant absorption peaks for heme c_1 (left) and heme b (right).

OCH_2), 7.69 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 7.76 (td, J = 7.6 Hz, J = 1.2 Hz, 1H, ArH), 8.09 (m, 1H, ArH), 8.12 (m, 1H, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Nonanoate (33). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 73% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 52.5−53.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.90 (t, 3H, J = 6.7 Hz, CH_3CH_2), 1.02 (s, 6H, 2 × CH_3), 1.28 (m, 8H, $(CH_2)_5CH_2$, 1.62 (m, 2H, $COCH_2CH_2$), 2.29 (t, 2H, J = 7.5 Hz, $COCH_2CH_2$), 2.71 (s, 2H, CH_2Ar), 3.87 (s, 2H, OCH_2), 7.47 (s, 1H, OH), 7.73 (t, 1H, J = 7.5 Hz, ArH), 7.80 (t, 1H, J = 7.6 Hz, ArH), 8.03 (d, 1H, J = 7.6 Hz, ArH), 8.06 (d, 1H, J = 7.6 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Decanoate (34). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow oil was obtained in 72% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 61.0−62.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (t, 3H, J = 6.8 Hz, CH_3CH_2), 1.02 (s, 6H, 2 × CH_3), 1.28 (m, 12H, $(CH_2)_6CH_2$), 1.62 (m, 2H, $COCH_2CH_2$), 2.29 (t,2H, J = 7.6 Hz, $COCH_2CH_2$), 2.71 (s, 2H, CH_2Ar), 3.88 (s, 2H, OCH $_2$), 7.46 (s, 1H, OH), 7.73 (dt, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 7.80 (dt, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.12 (d, 1H, J = 7.6 Hz, ArH), 8.15 (d, 1H, J = 7.6 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Undecanoate (35). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow solid was obtained in 83% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 64.5−65.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.90 (t, 3H, J = 6.8 Hz, CH_3CH_2), 1.01 (s, 6H, 2 × CH_3), 1.28 (m, 12H, $(CH_2)_7CH_2$), 1.62 (quin, 2H, $COCH_2CH_2CH_2$), 2.29 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 2.71 (s, 2H, CH_2Ar), 3.88 (s, 2H, OCH_2), 7.46 (s, 1H, OH), 7.73 (td, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH), 7.80 (td, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH), 8.12 (dd, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH), 8.15 (dd, 1H, J = 7.6 Hz, J = 1.1 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Dodecanoate (36). Flash column chromatography was used, eluting with 3% ethyl acetate−hexane. The yellow solid was obtained in 79% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 68.0−69.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.91 (t, 3H, J = 6.8 Hz, CH_3CH_2), 1.02 (s, 6H, 2 × CH_3), 1.28 (m, 14H, $(CH_2)_7$), 1.61 (m, 2H, $COCH_2CH_2$), 2.29 (t, 2H, J = 7.6 Hz, $COCH_2CH_2$), 2.71 (s, 2H, CH_2Ar), 3.88 (s, 2H, OCH_2), 7.46 (s, 1H, OH), 7.73 (td, 1H, J = 7.6 Hz, J = 1.3 Hz, ArH), 7.80 (td, 1H, J = 7.6 Hz, J = 1.3 Hz, ArH), 8.13 (dd, 1H, J = 7.6 Hz, J = 1.3 Hz, ArH), 8.16 (dd, 1H, J = 7.6 Hz, J = 1.3 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Tetradecanoate (37). Flash column chromatography was used, eluting with 4% ethyl acetate−hexane. The yellow solid was obtained in 80% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 79.0−80.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, 3H, J = 6.8 Hz, CH_3CH_2), 0.99 (s, 6H, 2 × CH_3), 1.25 (m, 20H, CH_3-$(CH_2)_{10}CH_2$), 1.59 (m, 2H, $COCH_2CH_2CH_2$), 2.26 (t, 2H, J = 7.6 Hz, CH_2CH_2CO), 2.68 (s, 2H, CH_2Ar), 3.86 (s, 2H, OCH_2), 7.44 (s, 1H, OH), 7.70 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 7.77 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.10 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.13 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Palmitate (38). Flash column chromatography was used, eluting with 4% ethyl acetate−hexane. The yellow solid was obtained in 77% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 76.0−77.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, 3H, J = 6.8 Hz, CH_3CH_2), 0.99 (s, 6H, 2 × CH_3), 1.25 (br s, 24H, CH_3-$(CH_2)_{12}CH_2$), 1.59 (m, 2H, CH_2CH_2CHCO), 2.26 (t, 2H, J = 7.6 Hz, CH_2CH_2CO), 2.68 (s, 2H, CH_2Ar), 3.86 (s, 2H, OCH_2), 7.45 (s, 1H, OH), 7.70 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 7.77 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.10 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.13 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)-2,2-dimethylpropyl Stearate (39). Flash column chromatography was used, eluting with 4% ethyl acetate−hexane. The yellow solid was obtained in 74% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 82.0−83.0 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88 (t, 3H, J = 6.8 Hz, CH_3CH_2), 0.99 (s, 6H, 2 × CH_3), 1.25 (br s, 28H, CH_3-$(CH_2)_{14}CH_2$), 1.59 (m, 2H, $CH_2CH_2CH_2CO$), 2.26 (t, 2H, J = 7.2 Hz, CH_2CH_2CO), 2.68 (s, 2H, CH_2Ar), 3.86 (s, 2H, OCH_2),

7.44 (s, 1H, OH), 7.70 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 7.77 (td, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), 8.10 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH), and 8.13 (dd, 1H, J = 7.6 Hz, J = 1.2 Hz, ArH).

General Procedure for Synthesis of Naphthoquinone Aliphatic Esters 42−44. A solution of 1,1′-carbonyldiimidazole (CDI) (0.32 mmol) in dry THF (3 mL) was added dropwise to the stirring and ice-cooled solution of carboxylic acid (0.32 mmol) in dry THF (1 mL). After being stirred for 4 h at room temperature, the reaction mixture was placed in an ice-cooled bath and to it was added dropwise a solution of 2-hydroxy-3-(3-hydroxypropyl)-1,4-naphthoquinone (0.22 mmol) in dry THF (1 mL) over 5 min. After being stirred at room temperature for 18 h, the reaction mixture was quenched with saturated ammonium chloride solution and extracted with dichloromethane (5 × 10 mL). The combined organic layer was washed with saturated sodium chloride solution (3 × 30 mL), dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography.

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)propyl Acetate (42). Flash column chromatography was used, eluting with 10% ethyl acetate−hexane. The yellow solid was obtained in 32% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 104.0−106.0 °C. ^{1}H NMR, (400 MHz, $CDCl_3$), δ: 8.15 (dd, 1H,ArH, J = 1.4, 7.5 Hz), 8.11 (dd, 1H, ArH, J = 1.4, 7.6 Hz), 7.79 (dt, 1H, ArH, J = 1.4, 7.5 Hz), 7.72 (dt, 1H, ArH, J = 1.4, 7.5 Hz), 7.40 (s, 1H, ArOH), 4.13 (t, 2H, $ArCH_2CH_2O$, J = 6.5 Hz), 2.72 (t, 1H, $ArCH_2$, J = 7.5 Hz), 2.06 (s, 3H, $O(CO)CH_3$), 1.95 (quint, 2H, $ArCH_2CH_2CH_2O$, J = 7.0 Hz).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)propyl Butyrate (43). Flash column chromatography was used, eluting with 10% ethyl acetate−hexane. The yellow solid was obtained in 60% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 77.0−78.0 °C. ^{1}H NMR, (400 MHz, $CDCl_3$), δ: 0.95 (t, 3H, $(CO)CH_2CH_2CH_3$, J= 7.5 Hz), 1.66 (sixt, 2H, $(CO)CH_2CH_2CH_3$, J = 7.5 Hz), 1.93 (quint, 2H, $ArCH_2CH_2CH_2O$, J = 6.5 Hz), 2.29 (t, 2H, $O(CO)CH_2CH_2CH_3$, J = 7.5 Hz), 2.72 (t, 2H, $ArCH_2CH_2CH_2O$, J = 7.5 Hz), 4.14 (t, 2H, $ArCH_2CH_2CH_2O$, J = 6.5 Hz), 7.39 (s, 1H, ArOH), 7.71 (dt, 1H, ArH, J = 1.4, 7.5 Hz), 7.78 (dt, 1H, ArH, J = 1.4, 7.5 Hz), 8.11 (dd, 1H, ArH, J = 1.4, 7.5 Hz), 8.14 (dd, 1H,ArH, J = 1.4, 7.5 Hz).

3-(1,4-Dihydro-2-hydroxy-1,4-dioxonaphthalen-3-yl)propyl Octanoate (44). Flash column chromatography was used, eluting with 10% ethyl acetate−hexane. The yellow solid was obtained in 49% yield, and then it was crystallized with hexane−dichloromethane to give yellow needles, mp 80.0−81.0 °C. ^{1}H NMR, (400 MHz, $CDCl_3$), δ: 0.89 (t, 3H, $(CO)CH_2(CH_2)_5CH_3$, J = 6.9 Hz), 1.29−1.70 (m, 10H, $(CO)CH_2(CH_2)_5CH_3$), 1.93 (quint, 2H, $ArCH_2CH_2CH_2O$, J = 7.0 Hz), 2.30 (t, 2H, $(CO)CH_2(CH_2)_5CH_3$, J = 7.6 Hz), 2.72 (t, 2H, $ArCH_2CH_2CH_2O$, J = 7.5 Hz), 4.14 (t, 2H, $ArCH_2CH_2CH_2O$, J = 6.5 Hz), 7.41 (s, 1H, ArOH), 7.72 (dt, 1H, ArH, J = 1.4, 7.6 Hz), 7.79 (dt, 1H, ArH, J = 1.4, 7.6 Hz), 8.11 (dd, 1H, ArH, J = 1.2, 7.6 Hz), 8.14 (dd, 1H,ArH, J = 1.0, 7.6 Hz).

(1-((3-Hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)methyl)-cyclohexyl)methyl Octanoate (46). A solution of compound **45** (0.177 mmol) in 1% aqueous sodium hydroxide solution (0.266 mmol) was refluxed for 1 h. After completion of the reaction, the reaction mixture was cooled to room temperature and neutralized with acetic acid to pH 7, then extracted with dichloromethane (3 × 15 mL). After concentration, the intermediate **10c** was obtained together with a small amount of the cyclized product **45** (monitoring by TLC). A solution of carboxylic acid (0.23 mmol), dicyclohexylcarbodiimide (DCC) (0.23 mmol), and DMAP (0.053 mmol) was stirred in dry CH_2Cl_2 (5 mL) at room temperature. After 3 h, a solution of compound **45** and naphthoquinone alcohol intermediate **10c** in THF (2 mL) was added and then the mixture was stirred at room temperature for 16 h. The precipitate of dicyclohexylurea was filtered off. The crude product was purified by flash column chromatography, eluting with 3% ethyl acetate−hexane to provide the desired product **46** (26.5 mg, 35% yield for two steps) as a yellow gum and cyclized product **45** (16.6 mg). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.81 (t, J = 3.4 Hz, 3H, CH_3), 1.10−1.92 (m, 20H, CH2), 2.34 (t, J = 7.56 Hz, 2H, COCH2), 2.65 (s, 2H, ArCH2), 3.91 (s, 2H, OCH2), 7.37 (s, 1H, OH), 7.62 (dt, J = 7.5, 1.4 Hz, 1H, ArH), 7.68 (dt, J = 7.5, 1.4 Hz, 1H, ArH), 8.01 (m, 1H, ArH), 8.04 (m, 1H, ArH).

FTIR, ^{13}C NMR, MS, and elemental analysis data of compounds **18−39**, **42−44**, and **46** are in Supporting Information.

Antimalarial Activity Assay. *Plasmodium falciparum* (K1, multidrug resistant strain) was cultivated in vitro according to Trager amd Jensen[13a] in RPMI 1640 medium containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 32 mM $NaHCO_3$, and 10% heat activated human serum with 3% erythrocytes and incubated at 37 °C in an incubator with 3% CO_2. Cultures were diluted with fresh media and erythrocytes everyday according to cell growth. Quantitative assessment of antimalarial activity in vitro was determined by microculture radioisotope techniques based upon the methods described by Desjardins et al.[13b] Briefly, a mixture of 200 μL of 1.5% erythrocytes with 1% parasitemia at the early ring stage was pre-exposed to 25 μL of the medium containing a test sample dissolved in 1% DMSO (0.1% final concentration) for 24 h, employing the incubation conditions described above. Subsequently, 25 μL of [^{3}H]hypoxanthine (Amersham) in culture medium (0.5 μCi) was added to each well and plates were incubated for an additional 24 h. Levels of incorporated radioactively labeled hypoxanthine indicating parasite growth were determined using a TopCount microplate scintillation counter (Packard). Inhibition concentration (IC_{50}) represents the concentration that indicates 50% reduction in parasite growth. The standard sample was dihydroartemisinin (DHA).

***Plasmodium falciparum* (Strain 3D7) and Yeast (*Saccharomyces cerevisiae*) Culture.** *P. falciparum* (strain 3D7) was cultured in a 2% O+ erythrocyte suspension as described in Biagini's method.[28] *S. cerevisiae* was grown in YPD broth at 28 °C as described in Fisher's method.[29]

Preparation of *P. falciparum* Cell-Free Extracts, Yeast Mitochondrial Membranes, and Rat Liver Microsomes. *P. falciparum* was harvested from infected erythrocytes as described in Biagini's method.[28] Parasite cell-free extracts were prepared immediately prior to use by 20 s of sonication on ice. Yeast mitochondrial membranes were prepared as described in Fisher's method.[29] Adult male Wistar rats were obtained from Charles River Laboratories (Margate, Kent, U.K.). Wistar rat liver microsomes were prepared from male rats (125−170 g) as described by Gill's method.[30]

Decylubiquinol Preparation. Decylubiquinol (2,3-dimethoxy-5-methyl-*n*-decyl-1,4-benzoquinol) was prepared by dithionite reduction of decylubiquinone (Sigma) as described in Fisher's method.[29]

Decylubiquinol/Cytochrome *c* Reductase Assay. Cytochrome bc_1 activity was monitored spectrophotometrically in *P. falciparum* cell-free extracts and yeast mitochondrial membrane preparations by the steady-state decylubiquinol/cytochrome *c* reductase using $\varepsilon_{550-542nm}$ in a Cary 4000 UV−vis spectrophotometer at 21 °C. Assays were performed in 50 mM potassium phosphate buffer (pH 7.5) containing 2 mM EDTA, 10 mM KCN, and 30 μM equine heart cytochrome *c* (Sigma) and the reactions initiated by the addition of 50 μM decylubiquinol. The total assay volume was 0.7 mL. Yeast and rat bc_1 concentration were assessed by the "dithionite reduced *minus* ferricyanide-oxidized" difference spectra, using the extinction coefficient constant $\varepsilon_{562-575nm}$ = 28.5 mM^{-1} cm^{-1},[15a] and the concentrations adjusted to 5 nM for the decylubiquinol/cytochrome *c* reductase assay. The presence of contaminating hemozoin precludes the use of spectrophotometric methods to directly determine cyt bc_1

concentration in *P. falciparum* cell-free extracts. Therefore, total protein concentration was first measured in the *P. falciparum* cell-free extract by the Bradford method[15b] and then adjusted to 0.1 mg protein/mL for the decylubiquinol/cytochrome *c* reductase assay. Compounds **31** and **37** were prepared as 20 mM stock solutions in DMSO and were added to the decylubiquinol/cytochrome *c* reductase system without prior incubation. The concentration of DMSO in the assay did not exceed 0.3% v/v. IC_{50} values were calculated using four-parameter sigmoidal curve fitting (Kaleidagraph, Synergy Software).

Protein Production and Purfication. Wild type yeast cells (strain CKWT) were grown in YPGal medium (1% yeast extract, 2% peptone, and 3% galactose) according to Wenz's method.[16] Yeast mitochondrial membrane preparation and cytochrome bc_1 complex (cyt bc_1) purification were described in Wenz's method.[31] The purified cyt bc_1 was stored in 50 mM potassium phosphate buffer (pH 7.5, including 300 mM NaCl, 250 mM sucrose, and 0.05% UM) at −80 °C. The cyt bc_1 was quantified by measuring the dithionite-reduced minus ferricyanite oxidized difference spectra at the α band of cyt bc_1. The extinction coefficient of *b* heme ($\varepsilon_{562-575nm} = 28.5\ mM^{-1}\ cm^{-1}$, was used to calculate the cyt bc_1 concentration.

The cyt bc_1 Activity Assay. The reaction buffer for ubiquinol−cytochrome *c* reductase activity of cyt bc_1 contained 50 mM potassium phosphate buffer, pH 7.4, 0.05% UM, 1 mM KCN, 0.2 mM EDTA, 250 mM sucrose, and 12 μM equine heart cytochrome *c*. Purified cyt bc_1 was diluted to 8 nM in the reaction buffer. Stigmatellin and **31** and **37** inhibitors were prepared as 40 and 20 mM stock solutions in DMSO, respectively. Reactions were initiated by adding decylubiquinol (DQH, final concentration of 50 μM) and monitored spectroscopically at 550 nm at room temperature. IC_{50} values were determined with a concentration series of stigmatellin and **31** and **37** compounds. Inhibitors were added to the reaction mixture prior to the start of the reaction.

Inhibitor Binding Assay. Cytochrome bc_1 was diluted to 2 μM in 50 mM potassium phosphate buffer containing 0.05% UM. Inhibitors were added to cyt bc_1 at a final concentration of 5 μM **31**, 5 μM **37**, 40 μM stigmatellin, and 40 μM antimycin A, respectively. Compounds **31** and **37** contribute absorbance between 500 and 600 nm; thus, 5 μM of both compounds was used to avoid interference with the absorbance of heme α and β bands. Redox difference spectra were recorded as DQH-reduced cyt bc_1−inhibitor complex minus protein−inhibitor complex.

Acknowledgment. This work was supported by the Thailand Research Fund (TRF), Thailand. N.P. and K.H. are Ph.D. students under the Royal Golden Jubilee Ph.D. program, TRF. Financial support from the Center for Innovation in Chemistry (PERCH-CIC) is also gratefully acknowledged.

Supporting Information Available: FTIR, ^{13}C NMR, MS, and elemental analysis data for compounds **18**−**39**, **42**−**44**, and **46**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Kongkathip, N.; Luangkamin, S.; Kongkathip, B.; Sangma, C.; Grigg, R.; Kongsaevee, P.; Prabpai, S.; Pradidphol, N.; Piyaviriyagul, S.; Siripong, P. Synthesis of Novel Rhinacanthins and Related Anticancer Naphthoquinone Esters. *J. Med. Chem.* **2004**, *47*, 4427–4438. (b) Kongkathip, N.; Kongkathip, B.; Siripong, P.; Sangma, C.; Luangkamin, S.; Niyomdecha, M.; Pattanapa, S.; Piyaviriyagul, S.; Kongsaeree, P. Potent Antitumor Activity of Synthetic 1,2-Naphthoquinones and 1,4-Naphthoquinones. *Bioorg. Med. Chem.* **2003**, *11* (14), 3179–3191.

(2) Rojanapa, W.; Tepsuwan, A.; Siripong, P. Mutagenicity and Antimutagenicity of Thai Medicinal Plants. *Basic Life Sci.* **1990**, *52*, 447–452.

(3) Fotie, J. Quinones and Malaria. *Anti-Infect. Agents Med. Chem.* **2006**, *5*, 357–366.

(4) Verma, R .P. Anti-Cancer Activities of 1,4-Naphthoquinones: A QSAR Study. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 489–499.

(5) Wendel, W. B. The Influence of Naphthoquinones upon the Respiratory and Carbohydrate Metabolism of Malarial Parasites. *Fed. Proc.* **1946**, *5*, 406–407.

(6) Fieser, L. F.; Berliner, E.; Bondhus, F. J.; Chang, F. C.; Dauben, W. G.; Ettlinger, M. G.; Fawaz, G.; Fields, M.; Fieser, M.; Heidelberger, C.; Heymann, H.; Seligman, A. M.; Vaughan, W. R.; Wilson, A. G.; Wilson, E.; Wu, M. I.; Leffler, M. T.; Hamlin, K. E.; Hathaway, R. J.; Matson, E. J.; Moore, E. E.; Moore, M. B.; Rapala, R. T.; Zaugg, H. E. Naphthoquinones. I−XVII. *J. Am. Chem. Soc.* **1948**, *70*, 3151–3244.

(7) Hudson, A. T. Atovaquone—A Novel Broad Spectrum Anti-infective Drug. *Parasitol. Today* **1993**, *9*, 66–68 and references therein .

(8) (a) Looareesuwan, S.; Chulay, J. D.; Canfield, C. J.; Hutchinson, D. B. Malarone (Atovaquone and Proguanil Hydrochloride): A Review of Its Clinical Development for Treatment of Malaria. Malarone Clinical Trials Study Group. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 533–541. (b) Baggish, A. L.; Hill, D. R. Antiparasitic Agent Atovaquone. *Antimicrob. Agents Chemother.* **2002**, *46*, 1163–1173.

(9) Kessl, J. J.; Lange, B. B.; Merbitz-Zahradnik, T.; Zwicker, K.; Hill, P.; Meunier, B.; Palsdottir, H.; Hunte, C.; Meshnick, S.; Trumpower, B. L. Molecular Basis for Atovaquone Binding to the Cytochrome bc_1 Complex. *J. Biol. Chem.* **2003**, *278*, 31312–31318.

(10) Mather, M. W.; Darrouzet, E.; Valkova-Valchanova, M.; Cooley, J. W.; McIntosh, M. T.; Daldal, F.; Vaidya, A. B. Uncovering the Molecular Mode of Action of the Antimalarial Drug Atovaquone Using a Bacterial System. *J. Biol. Chem.* **2005**, *280*, 27458–27465.

(11) (a) Hammond, D. J.; Burchell, J. R.; Pudney, M. Inhibition of Pyrimidine Biosynthesis de Novo in *Plasmodium falciparum* by 2-(4-*t*-Butylcyclohexyl)-3-hydroxy-1,4-naphthoqinone in Vitro. *Mol. Biochem. Parasitol.* **1985**, *14*, 97–109. (b) Fry, M.; Pudney, M. Site of Action of the Antimalarial hydroxynaphthoquinone, 2-[trans-4-(4′-Chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone (566C80). *Biochem. Pharmacol.* **1992**, *43*, 1545–1553.

(12) Wu, T. S.; Hsu, H. C.; Wu, P. L.; Leu, Y. L.; Chan, Y. Y.; Chern, C. Y.; Yeh, M. Y.; Tien, H. J. Naphthoquinone Esters from the Root of *Rhinacanthus nasutus*. *Chem. Pharm. Bull.* **1998**, *46*, 413–418.

(13) (a) Trager, W.; Jensen, J. B. Human Malaria Parasites in Continuous Culture. *Science* **1976**, *193*, 673–675. (b) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative Assessment of Antimalarial Activity in Vitro by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(14) Skehan, P.; Storeng, R.; Scudiero, D.; Monko, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. *J. Natl. Cancer. Inst.* **1990**, *82*, 1107–1112.

(15) (a) Vanneste, W. H. Molecular Proportion of the Fixed Cytochrome Components of the Respiratory Chain of Keilin−Hartree Particles and Beef Heart Mitochondria. *Biochim. Biophys. Acta* **1966**, *113* (1), 175–178. (b) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein−Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(16) Wenz, T.; Covian, R.; Hellwig, P.; MacMillan, F.; Meunier, B.; Trumpower, B. L.; Hunte, C. Mutational Analysis of Cytochrome *b* at the Ubiquinol Oxidation Site of Yeast Complex III. *J. Biol. Chem.* **2007**, *282*, 3977–3988.

(17) von Jagow, G.; Link, Th. A. Use of Specific Inhibitors on the Mitochondrial bc_1 Complex. *Method. Enzymol.* **1986**, *126*, 253–271.

(18) Fisher, N.; Meunier, B. Molecular Basis of Resistance to Cytochrome bc_1 Inhibitors. *FEMS Yeast Res.* **2008**, *8*, 183–8192.

(19) Zhao, P.-L.; Wang, L.; Zu, X.-L.; Huang, X.; Zhau, C.-G.; Wu, J.-W.; Yang, G.-F. Subnanomolar Inhibitor of Cytochrome bc_1 Complex Designed by Optimizing Interaction with Conformationally Flexible Residues. *J. Am. Chem. Soc.* **2009**, DOI: 10.1021/ja905756c.

(20) Altmann, K.-H.; Carreira, E. M. Unravelling a Molecular Target of Macrolides. *Nat. Chem. Biol.* **2008**, *4*, 388–389.

(21) Crowley, P. J.; Berry, E. A.; Cromartie, T.; Daldal, F.; Godfrey, C. R. A.; Lee, D.-W.; Philips, J. E.; Taylor, A.; Viner, R. The Role of Molecular Modelling in the Design of Analogues of the Fungicidal Natural Products Crocacins A and D. *Bioorg. Med. Chem.* **2008**, *16*, 10345–10355.

(22) Xiang, H.; McSurdy-Freed, J.; Moorthy, G. S.; Hugger, E.; Bambal, R.; Hau, C.; Ferrer, S.; Gargallo, D.; Davis, C. B. Preclinical Drug Metabolism and Pharmacokinetic Evaluation of

GW844520, a Novel Anti-Malarial Mitochondrial Electron Transport Inhibitor. *J. Pharm. Sci.* **2006**, *95*, 2657–2672.
(23) Yeates, C. L.; Batchelor, J. F.; Capon, E. C.; Cheeseman, N. J.; Fry, M.; Hudson, A. T.; Pudney, M.; Trimming, H.; Woolven, J.; Bueno, J. M.; Chicharro, J.; Fernandez, E.; Fiandor, J. M.; Gargallo-Viola, D.; de las Heras, F. G.; Herreros, E.; Leon, M. L. Synthesis and Structure−Activity Relationships of 4-Pyridones as Potential Antimalarials. *J. Med. Chem.* **2008**, *51*, 2845–2852.
(24) Djimdé, A.; Doumbo, O. K.; Cortese, J. F.; Kayentao, K.; Doumbo, S.; Diourté, Y.; Dicko, A.; Su, X.-Z.; Nomura, T.; Fidock, D. A.; Wellems, T. E.; Plowe, C. V. A Molecular Marker for Chloroquine-Resistant Falciparum Malaria. *N. Engl. J. Med.* **2001**, *344*, 257–263.
(25) Cowman, A. F.; Morry, M. J.; Biggs, B. A.; Crosst, G. A. M.; Foote, S. J. Amino Acid Changes Linked to Pyrimethamine Resistance in the Dihydrofolate Reductase−Thymidylate Synthase Gene of *Plasmodium falciparum*. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *85*, 9109–9113.
(26) White, N. J. Preventing Antimalarial Drug Resistance through Combinations. *Drug Resist. Updates* **1998**, *1*, 3–9.
(27) Kongkathip, N.; Kongkathip, B.; Hasitapan, K. Novel Antimalarial 1,4-Naphthoquinone Aliphatic Ester Derivatives with Inhibition of *Plasmodium falciparum*. Thailand Patent Application 0701003412, **2007**.
(28) Biagini, G. A.; Fisher, N.; Berry, N.; Stocks, P. A.; Meunier, B.; Williams, D. P.; Bonar-Law, R.; Bray, P. G.; Owen, A.; O'Neill, P. M.; Ward, S. A. Acridinediones: Selective and Potent Inhibitors of the Malaria Parasite Mitochondrial bc_1 Complex. *Mol. Pharmacol.* **2008**, *73*, 1347–1355.
(29) Fisher, N.; Castleden, C. K.; Bourges, I.; Brasseur, G.; Dujardin, G.; Meunier, B. Human Disease-Related Mutations in Cytochrome *b* Studied in Yeast. *J. Biol. Chem.* **2004**, *279*, 12951–12958.
(30) Gill, H. J.; Tingle, M. D.; Park, B. K. *N*-Hydroxylation of Dapsone by Multiple Enzymes of Cytochrome P450: Implications for Inhibition of Haemotoxicity. *Br. J. Clin. Pharmacol.* **1995**, *40*, 531–538.
(31) Wenz, T.; Hellwig, P.; MacMillan, F.; Meunier, B.; Hunte, C. Probing the Role of E272 in Quinol Oxidation of Mitochondrial Complex III. *Biochemistry* **2006**, *45*, 9042–9052.